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GENETICS AND MARKERS OF NAVICULAR DISEASE IN WARMBLOOD SPORT HORSES

DIPLOMA WORK

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CONTENT

1. INTRODUCTION

Ever since the horse was domesticated between five and six thousand years ago, the horse has been of great importance to humans in many different aspects of their life. From transportation and farming, to the use in warfare and military where the horse had a great impact on the outcome of battles. The search for healthy horses with desired qualities has been in focus of the breeding long before the knowledge about genomics and inheritance was known to horse breeders. Today the use of horses is mostly for sport and leisure and use of genetics are more employed in the breeding of horses.

Many trait qualities are genetically inherited in the horse, and more knowledge about this could be beneficial in the breeding of better quality horses. Desired colors and conformation, improved performance traits and excluding diseases that are carried with the genes, are all traits in different fields where the genomics can be used by an equine clinician. In 1995 a group of veterinarians and equine geneticists from 12 countries and 22 laboratories formed The Horse Genome Project to work toward performing large-scale studies of the equine genome. Their work has been of major importance and based on microsattelites they have been able to make several successful gene maps. In 2006 the horses was chosen by the US National Human Genome Research Institute of the National Institute of Health to get the complete genome sequenced. They managed to determine 85-95% of the genome, leaving only some highly polymorphic genes of the immune system un-sequenced. The sequence had coverage of 6,8x, which is a higher coverage than has been achieved for other mammalian species except the mouse and human. The genomic data has been made accessible for the public in special databases so further research will be carried out for years to come.

Due to the work carried out by the horse genome project 11 mutations causing 10 clinical syndromes have been identified, and commercially testing is available for all but one. This have been taken in to use by the breeders by identify carriers and confirm clinical cases, and by this the carriers can be removed from breeding. These monogenic traits Include Hyperkalemic periodic paralysis, Polysaccharide storage myopathy, Malignant hyperthermia, Glycogen branching enzyme deficiency, severe combined immunodeficiency, Junctional epidermolysis bullosa, Hereditary equine regional dermal asthenia, Overo lethal white

syndrome, Grey horse melanoma and Lavender foal syndrome. (BROSNAHAN, BROOKS, ANTCZAK, 2010)

Polygenic diseases are diseases involving both genetics and environmental factors and many of them are under research today. Before the Whole Genome Sequence these projects could not have been done, but today several commonly occurring diseases can be successfully linked to genes. One of these is navicular disease, which are currently under research by the University of Hanover in Germany in Warmblood horses as a part of The Horse Genome Project. There has for a long time been suspected to be a hereditary background of navicular disease, and research and articles about hereditary of navicular disease was published as early as 1986 by the University and Utrecht. After the horse genome was sequenced the search continues to find the genes involved in navicular disease.

Navicular disease is a complex disease of the podotrochlear apparatus of the horse. It is a chronic degenerative process including the navicular bone, navicular bursa, deep digital flexor tendon (DDFT) and collateral ligaments of the distal interphalangeal joint. It is a common cause of forelimb lameness. The disease has a genetic background and the study into this disease may help decreasing the prevalence in the horse population.

2. MATERIAL AND METHODS

The aim of the study is to give an overview of the genetic background of navicular disease in equine warmblood horses, since it is a common and important disorder in sports horses today. I have focus on main features of this disorder as well as give an overview of aetiology, diagnosing, treatment and heritability. New possibilities to manage breeding selection was developed when new molecular method has been explored, these include techniques such as whole genome scans, candidate gene analysis and SNP microarrays. I will also give a resume to the use of this knowledge in the breeding associations today.

Data for this study have mostly been collected from various books, articles and internet sources

3. NAVICULAR DISEASE

3.1 Background

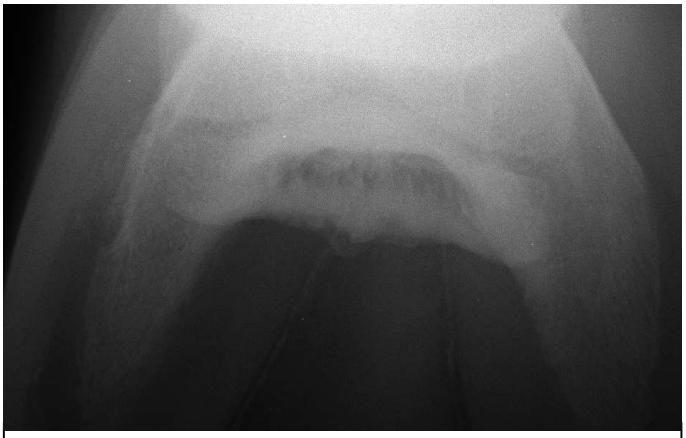
Navicular disease is a complex disease involving the whole podotrochlear apparatus leading to chronic forelimb lameness; very rarely it occurs in the hind leg. The navicular bone, the deep digital flexor tendon (DDFT), collateral sesamoid ligaments (CSLs), distal sesamoid impar ligament (DSIL) and the navicular bursa are all considered to be a part of the podotrochlear apparatus and degenerative changes in these structures considered as navicular disease. The disease can be acute or progressive and typically clinical signs do not appear until around 7-9 years of age when a decreased performance and intermittent forelimb lameness is noticed, or acute unilateral lameness. Lesions in the different structures are commonly appearing before any signs of pain have been detected.

The etiology of navicular disease is not yet fully understood. Identifying biochemical and genetic factors responsible for causing changes in the podotrochlear apparatus are important future topics that need to be searched for. Understanding what cause the pain and thereby clinical signs can help find correct preventive measures and optimum treatment. Today there are several theories of what causes the disease but they are usually divided into three main groups; vascular changes, pressure from DDFT and a process similar to osteoarthritis. In the vascular etiology theory it is explained that thrombosis of the distal arterioles of the navicular bone causes ischemia and necrosis, and it's from there the pain arrives. Thrombosis of the vessels leads to anostomosis and increased number of vessels, and the increased vascularisation leads to osteoporosis. Ischemia and thrombosis in the vessels have yet not been found or proven, and many researches have found hyperemia instead of necrosis in the area. For the second theory the continuous pressure from the DDFT to the flexor surface of the navicular bone is believed to cause degenerative changes or remodeling of the spongiosa. The pressure may also cause bursitis and decalcification of the navicular bone. To this theory there are several predisposing factors that influence the pressure of the DDFT such as poor shoeing with short heel and long toe, small hoofs and upright hoof conformation which will place extra pressure on the tendon. The third theory suggests degenerative changes similar to what happens in degenerative joint disease. Excessive, continuous pressure to the distal half of the navicular bone is suspected being the cause. Changed conformation of the foot may

play a contributing role giving a biomechanical stress to the navicular bone. Change in the fibrocartilage of the flexor surface and later the subchondral bone and marrow leads to edema and venous hypertension making, it a painful process.

3.2 Diagnosing

The disease usually presents as unilateral forelimb lameness, but can be bilateral as well. The clinical signs can vary and is sometimes not easy to detect at all, unless you are familiar with the normal stride of the horse. The horse might have resting pain, pointing one foot or alternating the front feet. The lameness or change in stride is often more obvious on hard ground on a circle, especially when the lame leg is inside the circle. The distal limb flexion test is very variable, but many horses will show an increased lameness. Often it can be increased with a wedge test. Some horses might respond to the hoof tester over the middle one third of the frog. None of the clinical signs are pathognomic for navicular disease.



Picture 1:Radiograph of the navicular bone in skyline view. Changed contour and inceased and enlarged canales

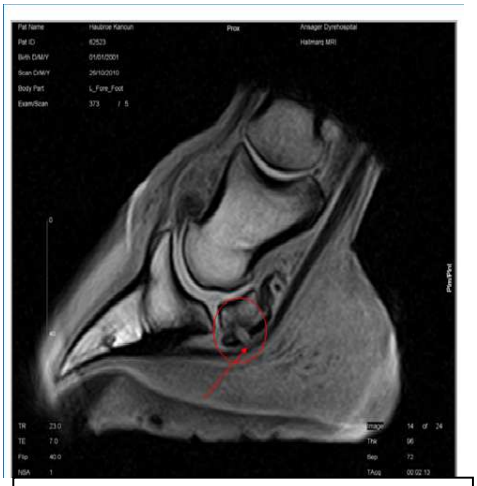
Positive response to analgesia of the distal interphalangeal joint and the navicular bursa is typically present in case of navicular disease, but not always. Analgesia of the palmar digital nerves often gives a decreased lameness, but does not go completely away. To diagnose navicular disease some diagnostic imaging is necessary. (ROSS, DYSON,)

Radiographic changes have been reported associated with navicular disease. Lateromedial (LM), dorsoproximal-palmarodistal oblique (DPr-PaDiO), palmaroproximal-palmarodistal oblique (PaPr-PaDiO) and flexed oblique images of the fetlock are of most use in the diagnosis. In the radiograph they look for the presence, number, shape, size and location of radiolucent zones along the distal border of the navicular bone; the presence of radiolucent zones along the proximal border of the navicular bone; medullary sclerosis; trabecular pattern

within the medulla; remodeling of the proximal border and extremities of the bone (enthesophyte formation) ; presence of mineralization within a CSL; presence of articular osteophytes; thickness of flexor cortex, radiolucent areas in the flexor cortex; new bone formation on the flexor surface; corticomedullary definition ; and presence of mineralized fragments distal to the navicular bone. There are not direct correlations between severity of radiographic changes and pain but certain changes are more commonly associated with clinical signs, and generally the more radiological changes detected in all projections the more probable it is of having clinical signs.

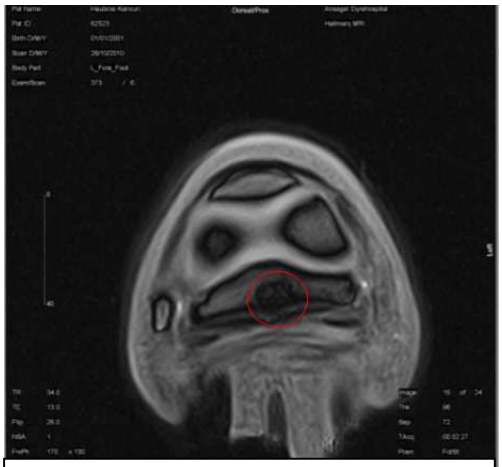
Nuclear scintigraphy detects bone turnover. Increased uptake has been seen in the navicular bone, DDFT, insertion of DDFT and distal phalanx in horses with foot pain in association with navicular disease. Positive scintigraphy is a good indicator of navicular disease, but a negative result does not exclude disease.

Ultrasound may also be used to detect navicular disease, using the frog as an acoustic window. In a study done by Grewald, McClure, Booth, Caston and Evans in 2004 they investigated the Assessment of the ultrasonographic characteristics of the podotrochlear apparatus in clinically normal horses and horses with navicular syndrome in 7 clinically normal horses and 28 horses with navicular syndrome. They were able to detect navicular bursitis, dystrophic mineralization of DDFT and impar ligament, tendonitis and insertional tendopathy of the DDFT and changes in flexor surface of the navicular bone with the ultrasound, and subtle dystrophic mineralization of DDFT could be easier detected than on radiological investigations. There were no objective measurements to prove the diagnosis, but significant subjective findings indicative of navicular disease is a useful method to diagnose the cause of the pain and characterize the different structures involved.



Picture 2: Inflammation of the navicular bone and adherence to the DDFT

Computer tomography is a sensitive method to detect changes in the podotrochlear apparatus similar to MRI.



Picture.3 : Inflammation of the navicular bone and adherence to the DDFT

Both will detect smaller changes and more structures than you can with x-ray, Ultrasound or scintigraphy.

MRI is a very sensitive method to detect lesions of the navicular bone and lesions in the structures of the podotrochlear apparatus. It has a high specificity for lesions in DDFT, CSLs navicular bursa and navicular bone. A study performed with 264 horses with unilateral or bilateral forelimb lameness showed that 82,6% of the limbs had lesions in DDFT most commonly at the level of CSLs (59,4%) and the navicular bone (59,0%). There was a positive association between DDFT lesions and navicular bone pathology involving all aspects of the bone. Lesions in DSIL (38,2%)were more common than CSLs (10,5%) but in either case they were associated with abnormalities in the navicular bone. (DYSON, MURRAY, 2007)

Endoscopic examination let you have a closer look at the fibrocartilage on the flexor surface of the navicular bone, the bursa, the dorsal surface of the DDFT and a limited view of the DSIL.

3.3 Treatment and management

Shoeing and trimming is a major part of the management of navicular disease. The aim is to correct any imbalance in the hoof and decrease the pressure on the navicular region. The break over point should be brought back and use of quarter clips instead of toe clips. Eggbar shoes, heartbar shoes and natural balance shoes have been suggested and improved the condition in some cases (SIMON CURTIS, 2002). For horses with broken back hoof pastern axis or severely collapsed heels a temporarily wedge or graduated shoe may be used to decrease the load on the DDFT.

For drugs Isosuxprine has often been used. It is a beta antagonist with the mode of action not fully understood, but have shown effective against navicular disease. It is an anti-inflammatory and hemorheological drug that will give a peripheral vasodilatation, and even in the non-vascular origin theory there might be a secondary congestion making the drug effective. After initial dose some horses may show clinical improvement, but usually there is need of indefinite medication. If there are no changes within 30 days there will usually be no effect of the drug. The response of the drug is less effective with major radiographic changes.

Other vasoactive drugs have also been used such as warfarin, pentoxifylline and metrenperone, but none have a proven effect. Injections into the distal interphalangeal joint with methylprednisolone acetate or triamcinolone acetonide may show temporarily improvement in horses with low grade lameness, but effectiveness is decreased when there are radiographic or MRI changes of the navicular bone. Due to the similarity of the disease with osteoarthritis a systemic administration of polysulphated glycosaminoglycan (PSGAG) has been tried, but no long-lasting benefits on lameness have been seen.

Non-steroidal anti-inflammatory drugs are commonly used, among them phenylbutazone most often. This will frequently take the pain away effectively, but the pain will usually come back when the horse is taken off the drug. For second choice Flunixin frequently used as NSAID. Tiludronate is a bisphosphate that inhibits osteoclastic activity. IV infusion of the Tiludronate to horses with navicular syndrome improves the lameness score, but has not proven full improvement in horses. Shockwave has alo been used as therapy and may have an improvement of chronic navicular disease (>3 months).

Palmar digital neurectomy is an option if no other treatments are successful. If one is able to remove the pain completely with the palmar neural analgesia and the lameness disappears it might give a pain-free life for the horse. The procedure does have a number of complications including neuroma and axon sprouting and the procedure should not be done if there are lesions of the DDFT when it may lead to rupture of the tendon. After neurectomies the horse can not be used in competitions.

Desmotomy of collateral sesamoidean ligaments, desmotomy of the accessory ligament of DDFT are also been described as alternative treatment with either short term improvement

(desmotomy of CSLs) or not well documented results. Chemical neurectomy with sarapin or absolute alcohol injected perineurally to the palmar digital nerves will cause temporarily loss of sensation for 2-3 months. Freezing the nerves percutaneus may also be used. This method have variable outcome and lameness usually return when the sensation comes back.

Decompression of the Navicular bone by surgical drilling is a new approach but long term follow up results are not yet available. The aim is to reduce intraosseous pressure by drilling cyst like lesions in the navicular bone.

Prognosis of navicular disease with clinical and radiological changes is generally poor. If there are a predisposing limb deformity it will be worse compared to a lameness of short duration with poor foot conformation. (DYSON, ROSS, 2011)

4. GENETICS OF NAVICULAR DISEASE

4.1 Current genetics

The last twenty years there have been many developments into the equine genome. The Horse Genome project that were started in 1995 and later they completed the whole genome scanning of the horse in 2009 that is of major importance for all genetic research in the field of equine genomics. It is the work done by these groups that made it possible to discover the monogenic traits, and their diagnostic tests that can prevent further spreading of these diseases. The current molecular genetic testing done on horses are either identification/parentage or disease/trait testing. It is based on polymerase chain reaction (PCR) for amplification of certain DNA targets. DNA test for monogenic traits are available at commercial laboratories and are frequently used today.

Further gene discovery projects and polygenetic traits are currently being investigated on the basis of the results and achievements of the whole genome scan and the Horse Genome project. Among these projects navicular syndrome is under investigation by the University of Hanover. Navicular disease is a polymorphic trait and therefore more difficult to characterize

since it consists of additive genetic effects of several genes and also environmental factors also play a part.

With the work of the Horse Genome Project there was developed several genetic maps that can map traits to chromosomes or sub chromosomal levels and later the gene sequence was developed. The equine maps that have been developed includes; radiation hybrid maps, comprehensive linkage maps, medium density horse gene map, high density microsattelite maps, informative marker set for QTL maps. A whole genome shotgun library was sequenced at the Broad Institute in Cambridge Massachusetts and Bac ends sequenced at the university of veterinary medicine in Hannover and Helmholtz centre for infection research. Over one million single nucleotide polymorphisms have been detected during the sequencing by the first horse with a whole genome scan and additional seven other breeds. The knowledge obtained in the scans is made available to the public, making it easier for future researches to discover new genes involved in equine diseases.

Quantitative trait loci (QTL) are genomic regions highly likely to contain genes influencing the traits of interest. To identify QTL responsible for certain diseases a high density linkage map of the whole genome with an equidistantly distribution are necessary. For the detection of QTLs responsible for navicular disease and many other equine diseases different genetic markers are in use today, but often in equine genetics microsattelite markers and single nucleotide polymorphism (SNP) have been most frequently used. The markers have to be polymorphic, evenly spaced and give dense marker coverage and low genotyping error rate. SNPs are common and evenly distributed in the genome, but have less variability per marker and will need more SNPs to provide the same information as microsattelites. Microsattelites, which can have more alleles, are able to cover a larger area of the genes that will be beneficial when starting to look for QTLs. SNPs are able to provide a denser map compared with microsattelite and are often used when the region in interest are identified.

With this further insight and methods of investigating the equine genome the search for polymorphic traits continuous all over the world. The draft of the gene sequence is now in place and details of the refinements are the next step in the research.

4.2 Navicular genetics

Survey of recent articles

As early as 1986 the University of Utrecht concluded that a familiar background of navicular disease could not be excluded on the basis of radiological and clinical examination of daughter groups of one group of 11 stallions. Since only a third of the foals were able to be included in the research a random sample was not possible, but the material collected detected a difference in frequency of abnormality in the navicular bone between progeny groups. Although further investigation had to be done, it supported the familiar background and that genetic factors are of importance.

4.2.1 Evaluation of expected response to selection for orthopedic health and performance traits in Hanoverian Warmblood horses.

5928 Hanoverian warmblood horses were used in the study by Stock and Distl in 2005 into the expected result to selection for orthopedic health and performance. Osseous fragments in the metacarpo-metatarso phalangeal (OFM), osseous fragments in the fetlock (OFF), deforming arthropathy in tarsal joints (DAT) and pathologic changes in the distal sesamoid bones (PCN) were chosen as the orthopedic disorders in question due to their high prevalence in the investigated horses.

The radiographs were done at the standard auction x-rays for the auction in Verden between 1991-2003 when the horses was at an average age of four years old, and the prevalence of PCN was found to be 24,7%. For the pedigree the information was provided by a unified horse ownership database with four generations included and a total of 23662 horses. Grandparents, parents and proband sires were used for the study. The breeding values were predicted multivariate in linear animal models using restricted maximum likelihood (REML). Relative breeding value for dressage and show jumping (TID, TIJ) published by Federation Equestre Nationale was used for the performance traits. The combinations of the breeding values used were total indices for dressage-radiographic findings (DR), jumping-radiographic findings (JR) and dressage-jumping-radiographic findings (DJR). Also selection for radiographic findings alone was investigated.

A completely orthopedic health selection gave an increase of RBV for orthopedic health, a decrease in prevalence by 17, 9%-31, 3 %. With selection for only one orthopedic health trait the prevalence decreased from 32%-54,7 % in the different orthopedic health traits. When selecting for DJR an increase in RBV of 4%-9,1 % and the prevalence of all but OFM decreased by 2,7%-15,8 %. For selection for only one riding trait DR or JR gave an increase in RBV for orthopedic health and the performance related total indices increased by 8%-8,5% for TID and DR and 16,3%-18,2 % for JR and TIJ but in this case the unconsidered traits (TIJ in JR and visa versa) decreased slightly. In a complete selection for performance one could detect stabilization or slight decrease of DAT and OFT findings, but PCN and OFM became considerably more prevalent. With an equal selection of both a slight decrease in the prevalence off all radiographic findings could be detected.

4.2.2 Genetic correlations between performance traits and radiographic findings in the limbs of German Warmblood riding horses

In a study done by Stock and Distl in 2007 they aimed to determine the genetic correlation between prevalent radiographic findings of the limb of young Warmblood riding horses and performance traits routinely evaluated in young horses of the same breed. Mare performance test (MPT) results were provided by the Association of Hanoverian Warmblood breeders between 1995 and 2004 for 12661 mares. They were judged for quality of gaits (walk, trot, and canter) under rider, jumping talent, rideability and character with scores from 0-10. Auction inspections for 4773 Hanoverian Warmblood horses was included, with scoring of quality of gaits under rider, jumping talents and rideability. During auction the horses for sale undergo clinical examination including radiograph of the limbs, here 5102 horses from 1997-2004 had radiographs with ten projections taken of the limbs and two radiologists evaluated them. They investigated osseous fragments of the fetlock (OFF), osseous fragments of hock (OFH) deforming arthropathy hock (DAH) and distinct radiographic findings in the navicular bone (DNB) as binary traits and additionally radiographic appearance of the navicular bone was investigated as a quasilinear trait with eight categories.

Pedigree information of all horses was included and made available by unified animal ownership database. Simple and multiple ANOVA were performed, the performance traits and RNB in GLM and mixed linear models and the binary traits in generalized linear model. The genetic parameters were estimated multivariatly in linear animal models with REML.

Prevalence for radiographic findings for DNB was 20, 13%, mean RNB score 0,69 ±1, 06 in all radiographed horses. For the radiographed horses with performance record the DNB prevalence was 21,21% and mean RNB score 0,71±1,04. The DNB prevalence and RNB score was influenced by date of presumable auction, year of examination, year of birth, sex, and sex X age interaction ($p<0,001$). Horses affected with DNB received an average lower score during the performance tests than horses not affected with DNB.

Heritability estimates for radiographic changes ranged between h^2= 0,05-0,11 before transformation and h^2=0,10-0,34 after transformation. Additive genetic correlation between performance traits and radiographic changes wee mostly close to zero.

The performance of young sports horses was expected to be higher when they were not genetically predisposed to develop radiographic changes in the limbs. So a selection for radiographic health is potential of high value to decrease radiological change associated lameness of horses, according to the findings of this study.

4.2.3 Genetic analyses of the radiographic appearance of the distal sesamoid bones in Hanoverian Warmblood horses

Stock, Distl and Habil investigated in 2006 whether additive genetic correlation existed between certain aspects of the radiographic appearance of the navicular bones (RNB) or between RNB and other types of radiographic changes in the limbs: osseous fragments in the fetlock joints (OFM), osseous fragments in the tarso crural joint (OFT) and deforming artropathy in the tarsal joints (DAT). 5157 Hannoveranian Warmblood horses were included in the research between 1997 and 2004, all clinically and radiographic examined by the same veterinarian and the pedigree data made available by an animal ownership database. The RNB was divided into seven categories ; I = few short and conical canalis sesamoidalis (CSs)

in the central portion of the distal border, II= several short and conical CSs in the central portion of the distal border , III= few elongated or deformed CS in the central portion n the distal border, IV= several elongated or deformed CS in the central portion of the distal border, V= markedly deformed CSs in the central, medial, or lateral portion of the distal border, VII = alteration in the structure of the navicular bone .The different categories were then used to define two quasilinear traits: RNB_{0-7} and RNB_{0-3} (3 = III-VII), and five binary traits: $RNB_{0/1a}$ (category I), $RNB_{0/1b}$ (category II), $RNB_{0/1c}$ (category III-VII), $RNB_{0/1d}$ (category II-VII) and $RNB_{0/1e}$ if they had any changes in the navicular bone. They were analyzed in general linear models using simple and multivariate ANOVAs. A multivariate analysis in linear animal model via restricted maximum likelihood was used to analyze genetic variables

The prevalence of horses with abnormal appearance of the navicular bone was 40, 57% and 98,66% of those horses the abnormal findings involved the distal border of the navicular bone. Males had a higher risk of having higher RNB values ($p<0,001$) except $RNB_{0/1a}$. Age was significantly related to all of the RNB traits where three years old mares were least likely to have RNB changes and 4 years old stallions had the highest risk. Heritability estimates for the RNB traits ranged from h^2= 0,10-0,34. For the quasilinear traits the heritability estimates were RNB_{0-7} h^2=0,133-0,156 and RNB_{0-3} h^2=0,179-0,193. For the binary traits there were larger standard errors for heritability than the quasilinear. $RNB_{0/1a}$ h^2=0,09-0,10 , $RNB_{0/1b}$ h^2=0,24-0,26 , $RNB_{0/1c}$ h^2=,0,16-0,19 , $RNB_{0/1d}$ h^2=0,22-0,25, $RNB_{0/1e}$, h^2=0,33-0,34.

Additive genetic correlation among those traits was close to unity. Negative additive correlation between osseus fragments in the fetlock (OFM) and $RNB_{0/1a}$, OFT and RNB_{0-7}, RNB_{0-3}, $RNB_{0/1a}$, $RNB_{0/1b}$, $RNB_{0/e}$, and between DAts and $RNB_{0/1a}$ and $RNB_{0/1e}$ (rg=-0, 40 to -0,11). Positive additive genetic correlation were estimated between OFMs and $RNB_{0/1e}$ and between DATs and $RNB_{0/1c}$ (rg= 0,19 to 0,25)

There was a low prevalence of radiographic findings fitting into five of the seven categories, so a larger number of horses would make a more reliable estimate. But from the study it showed that the heritability estimates were moderate except in category I, but this change in the navicular bone might be considered to be a physiological appearance of the bone. The

study could also show that few short and conical CS in the central portion of the distal border of the navicular bone were less likely to occur in horses affected with OFMs, OFTs or DAT.

4.2.4 Genetic correlation between conformation traits and radiographic findings in the limbs of German Warmblood riding horses.

Stock and Distl investigated in 2006 the correlation between conformation trait and radiological findings in the limb. Studbook inspection of 20768 mares divided into 14 traits, and radiographic results from 5102 Hannoveranian warmblood horses were used in the study. The five radiographic changes investigated was osseous fragments in the Fetlock (OFF) and hock (OFH), deforming arthropathy in hock joints (DAH), distinct radiographic findings in the navicular bones (DNB) and radiographic appearance of navicular bones (RNB). The four first ones were analyzed as binary traits while RNB as quasi linear traits. The radiographic changes were considered as all or none.

Conformation data from studbook inspections (SBI) was taken from mares between 1992 and 2001 and included 15 traits. Wither height, conformation and basic quality of gates. Head, neck, saddle bearing area, conformation front leg, conformation hind leg, frame and general impression and development with a score from 1-10. Walk at hand, correctness of gaits in walk and trot, impetus and elasticity in walk and trot.

The genetic parameters were estimated multivariatly in linear animal model with REML. SBI traits and RNB was analyzed in general and mixed models using GLM and MIXED procedures while the binary traits were analyzed in generalized linear models using GENMOD procedures.

The prevalence of radiographic findings in the 5102 radiographed horses were for DNB 20,13% and mean RNB score was 0,69. Heritability estimation for the binary RR traits were h^2=0,11 for DNB and h^2=0,22 after transformation and for RNB h^2=0,15. Positive additive correlation between SBI and RR in a range of rg= 0,15-0,52 was estimated between RNB and correctness of gaits, walk at hand and impetus and elasticity and between DNB and correctness of gaits. Negative additive effect in a range of rg=-0,15 to -0,56 were estimated between both DNB and RNB and conformation of hind leg .

Both traits involving the radiographic appearance of the navicular bone showed a positive genetic correlation to the SBI traits concerning gaits, but no correlation was detected to conformation of front limbs.

4.2.5 Multiple-trait selection for radiographic health of the limbs, conformation and performance in Warmblood riding horses

In 2008 Stock and Distl wanted to identify the mode of sire selection with which maximum breeding progress according to important conformation, performance and radiographic health traits can be achieved in the Warmblood riding horse. Radiographic data from 5155 Hannoveranian Warmblood horses, mostly between three and four years old, were included in the study and the x-rays were evaluated by two experienced veterinary radiologists. They found as the most frequent findings; osseous fragments in the fetlock (OFF), osseous fragments in the hock (OFH), deforming arthropathy hock (DAH), and distinct radiographic findings in the navicular bone (DNB), and were in this study all coded as binary traits. Conformation data were provided by the Hannoveranian Society of mares presented to studbook between 1995 and 2004 and conformation score on front limb, hind limb ad withers height was included. 16098 performance evaluated horses from results from auction inspection including quality of walk, trot, canter, under rider, ability and style of free jumping, rideability and character. Pedigree of all horses was provided by the unified animal ownership database, including seven ancestral generations of the horses in the analysis. The genetic parameters were estimated with the use of multivarietly in linear animal models with restricted maximum likelihood (REML).

The prevalence of DNB was in this study found to be 20,10% . The heritability estimates for the radiographic changes ranged between h^2=0,15-0,35, and for DNB h^2=0,23±0,03. The additive genetic correlation was in many cases close to zero, but moderate negative additive correlation between conformation hind leg and DNB (-0,53 to -0,16). Single trait selection on DNB reduced the prevalence of the trait with 37%, and selection for all radiographic changes reduced the prevalence between 14 % and 29%. Selection for all radiographic changes and conformation, performance or both reduced the prevalence by 11-20%. In this case the mean score for conformation and performance increased by maximally 4%.

4.3 Heritability

By calculating the heritability one will find out if the trait in question is hereditary and if it is possible to select the genotype away. The heritability estimates for navicular disease have been estimated to be between h^2= 0, 1 and h^2= 0, 34 and with a low to moderate heritability a genetic improvement should be possible to obtain with breeding, but the process will be slow. By studies it is shown that warmblood horses, quarter horses and are more prone to the disease, and deeper studies are done in warmblood horses.

Table 1: Heritability estimates in warmblood horses

Author, year	Number of horses	Heritability estimates	**breed**
Stock, Distl, Habil 2005	5157	0,10-0,34 (RNB)	Hanoverian warmblood
Stock and Distl 2006	5102	0,11 0,22 (DNB) 0,15 (RNB)	Hanoverian warmblood
Stock and Distl 2007	5102	0,10-0,34	Hanoverian Warmblood
Willms et al 1999	472 220	0,20-0,31 (mare) 0,20-0,25 (foal)	Holsteiner Warmblood
nStock and Distl 2008	5155	0,20-0,26	Hanoverian warmblood
KWPN 1994	590	0,26-0,32	Dutch warmblood

Heritability estimates for radiographic changes of the navicular bone in different research done on warmblood horses are in the range of h^2=0,10 to h^2=0,34. KWPN estimated in 1994 the heritability of radiological visible changes in the navicular bone based on 590 mares by

30 stallions to be h^2=0,26-0,32. Later in 1999 Willms et al estimated the heritability of the same criteria to be h^2=0,20-0,31 for 472 mares by 97 stallions and h^2=0,20-0,25 in 220 Holsteiner Warmblood foals.

Stock and Distl found in 2005 the heritability of radiographic changes in the Navicular bone to be between h^2=0,10 and 0,34 (LAM, quasilinear and binary traits). They went into detail to find the different degrees of changes and their heritability estimates and found that the genetic bases for different types of radiographic changes in the navicular bone were not identical, but mild changes seem to be less heritable; h^2= 0,09-0,10 for mild changes (only a few short and conical canalis sesamoidalis (CS) in the central portion of the distal border), h^2=0,24-0,26 for moderate changes (several short and conical CS in the central portion of the distal border), h^2=0,16-0,19 for severe changes (few elongated or deformed CS in the central portion n the distal border, several elongated or deformed CS in the central portion of the distal border for markedly deformed CS in the central, medial, or lateral portion of the distal border or alteration in the structure of the navicular bone) h^2=0,22-0,25 for moderate to severe and h^2=0,33-0,34 for changes in the distal border of the navicular bone.

The research done by Stock and Distl in 2006 found a prevalence of 20, 13% of DNB and heritability of distinct radiographic findings in the navicular bone was h^2= 0, 22 (LAM, binary) and for radiographic changes in the navicular bone h^2= 0,15 (LAM, quasilinear). In 2007 the heritability estimate was found to be in the range of 0,10-0,34 for several radiographic changes estimated (LAM,) In 2008 Stock and Distl investigated 5155 Hannoveranian Warmblood horses and found the heritability estimates for the radiographic changes ranged between 0,15-0,35 and for distinct radiographic changes in the navicular bone h^2=0,23±0,03.

4.4 QTLs and microsattelite markers

After the whole genome scan of the horse was done and a microsattelite marker set developed it became easier to search for QTLs. Diesterbeck, Hertsch and Distl did in 2007 a genome wide search for microsatellite markers associated with radiological alterations in the navicular bone of Hanoverian Warmblood horses. The aim of the study was to identify Quantitative trait loci (QTLs) for pathological changes in the navicular bone in Hanoverian Warmblood horses. The radiological changes investigated in the study were deformed canals sesamoidales, changed radiographic contour and structure of the navicular bone, which are traits significantly associated with development of navicular disease.

A whole genome scan of 192 horses in total, progeny and grandchildren of seventeen Hanoverian Warmblood stallions, were performed. All were radiographic examined, most of them between two and six years old, and blood and hair samples collected. DoPr-PaDi approach of the radiographs were taken and classified according to Brunken (1986). The findings were organized into binary traits where C1, C2a and C2b (unaffected based on canalis sesamoidalis), S1a and S1b (unaffected according to structure) and K1 (unaffected according to contour). Horses with grades C3a up to C6 and CP were determined to have to many short, elongated or deformed canales sesamoidales (DCS), S2a up to S4a had radiologically visible alteration of the structures of the navicular bone (RAS) and K2 to K5 had radiologically visible changes in the contour of the navicular bone(RES).

161 polymorphic microsattelite markers taken from published equine maps and HORSEMAP database at INRA Biotechnology Laboratories home page. The microsattelite markers were chosen to cover the entire equine genome with an evenly distribution. The equine chromosomes with highest LOD scores and Z means from the multipoint non-parametric linkage analysis (using Merlin software) were then selected and an increased density of microsattelite markers added. On equine chromosome 2 (ECA2) 23 microsattelite markers, on ECA3 8 microsattelite markers, 13 microsattelite markers on ECA10 and 8 microsattelite markers on ECA15 were added in regions that were assumed to have QTLs with multipoint chromosome-wide error probabilities ($p \leq 0.05$) for the linkage test statistics. They estimated the linkage between the microsattelite markers and radiological findings through the proportions of alleles shared identical by descent (IBD) and error probabilities calculated.

Chromosome wide significant QTLs were found on five different chromosomes; ECA 2, 3, 4, 10 and 26, and suggest that several genes may be responsible for navicular disease. Chromosome wide significance for DCS was found on ECA3 at 30,2cM (SG18) to 34,0cM (UMNe158) and on ECA10 at 4,8cM (HMS023) and 45,5 (SG30) to 55,0cM (LEX017). Chromosome wide significance for RAC was found on ECA2 at 48 (UCD380) to 62,2cM (THY340), on ECA3 at 0,0cM (AHT036), on ECA4 between 71,01cM (ASB22) and 73,3cM (HTG019) and on ECA26 at 6,9cM (COR071). Genome wide significant QTLs based on LOD scores reaching the treshold of the genome wide error probability for RAC was found between 48 and 62,2 cM on ECA 2 ($p<0,01$) and for DCS on ECA 10 from 45,5 to 49,8 cM.($p=0,02$).

Lopes, Diesterbeck, da Camara Machado and Distl followed the previous study in 2007 and their aim was to fine map the QTLs on ECA2 associated with navicular disease. 192 Hanoverian Warmblood horses were used in the study. They wanted the previous detected QTL involved in navicular disease refined as a further step to develop a marker test for the disease. A total of 58 microsattelites were used covering the chromosome with an average spacing of 2,1 Mb, 18 previously published microsattelite markers and nine newly developed were added.

A genome wide significant linkage for RAC could be shown for the microsattelite ABGe342 at 34,42 Mb. The nearby marker ABGe343 at 35,23 Mb reached a genome wide significance level of P = 0,064 as the highest Z mean and LOD score. Chromosome-wide significant linkage with RAC was achieved for the markers at 29,47 Mb ABGe007, at 32,50-43,13Mb (UCDEQ380-TKY2643) at 46.26 Mb (ABGe346) and a new QTL for RAC at 59,08-65,14 Mb (AHT012-HMS016). Significant genotypic and allelic associations as well as haplotype trait associations (two to four markers included) with RAC were found for markers at 31.99-42.56 Mb, 52,42Mb and 59.08-70.51 Mb.

Lopes, Diesterbeck, da Camara Machado and Distl also did, based on the previous study from 2007 when they discovered the QTLs associated with navicular disease, a study to refine the quantitative trait loci on equine chromosome 10 for radiological signs of navicular disease. 192 Hanoverian Warmblood horses were used in the study in 2010. The aim of the study was to refine the QTL for DCS that was previously discovered to be at 4,8cm (HMS23)

and at 45,5 (SG30) to 55,0 cm (LEX017) on ECA10 using a dense and informative marker set. This QTL was selected due to the high test statistics compared to other QTLs in the whole genome scan. The previously identified QTL is mapped to ECA10 at 9,99Mb and 14,45-41,43 Mb. For this study they increased the number of markers from 21 to 45 on ECA10 to get a dense coverage. Four previously published microsattelites, seven new microsattelites and thirteen SNPs. The average distance of the markers within the QTL were 0,64 and 1,29 Mb. With this they were able to confirm two QTL for DCS on ECA10 at 9,99 Mb and 14,45-41,43Mb. Nine microsattelites and three SNPs reach the highest Z mean and LOD score at 19,34-20,38 Mb and 23,17-30,73 Mb ($p<0,05$), but no indications for further QTL for DCS when he LOD score as and Z means were not of high enough genomic wide significant levels. The average polymorphism information content (PIC) of the marker set was 48,45 % ranging from 8,66% to 83,43% and the mean observed heterozygosity was 54,28% with a maximum of 85,64 and minimum 9,52%.

4.5 Candidate genes:

There are yet no confirmed candidate genes located that are responsible for navicular disease in horse, but some candidate genes are highly suggestive to be involved in the disease and should be looked into to clarify their role. The use of candidate genes to DNA test for diseases can be difficult when it is a high number of candidate genes involved in the syndrome, but the investigation into these genes are also important in the understanding of the disease and possible selection of healthy horses.

Some candidate genes presumed involved in navicular disease has been detected on the basis of Diesterbeck, Hertsch and Distl study in 2007 when they discovered five QTLs for navicular disease. Previous studies done by Svalstoga indicate that navicular disease resembles human osteoarthritis, and human molecular genetics have discovered candidate genes for OA in humans. The QTLs found in the study that are linked to navicular disease were homologous to human genomic regions linked to OA.

On ECA 2 at 48.0 cm COL16A1 (coding for alpha chain of type XVI collagen), MATN1 (matrilin 1 cartilage matrix protein), and ALPL (alkaline phosphatase). On ECA 3 at 0cm and

30.20 cm Matrix metalloproteinase 2 and 15 (MMP-2 and MMP-15), also on ECA 3cadherin 11 (CDH11) is a possible candidate gene. On ECA 4 COL1A2 (alpha 2 type I collagen), BBS9 (Bardet-Biedl syndrome 9, parathyroid hormone-responsive B1), and BMPER (bone morphogenetic protein-binding endothelial receptor).

On ECA 10 the SNP within the candidate genes IRF3 (19,44Mb) there was a significant allelic association with DCS and VSTM1 (23,06 Mb) a significant genotypic association with DCS were in linkage equilibrium with DCS and could be important for further investigation. They found potential candidate genes within the QTL on collagen type XII, alpha1 (COL12A1), myeloid-associated differentiation marker (MYADM), osteoclast associated immunoglobulin-like receptor (OSCAR), interleukin 11 (IL11), and collagen type X alpha1 (COL10A1).

In the study on ECA 2 they discovered suitable candidate genes. Alkiline Phosphatase(ALPL), tumor necrosis factor superfamily member1 B (TNFRSF1B), natriuretic pepide precursor B (NPPB), stathmin 1/oncoprotein 18 (STMN1) and WD repeat domain 8 (WDR8) located at 29,51-46,47 Mb but need further investigation.

From these studies we have some possible candidate genes involved in navicular disease, but further research into the topic are needed.

5. CURRENT SELECTION FOR HEREDITARY ORTHOPEDIC HEALTH

Different breeding associations around the world have different requirement for the approval of horses for stallion licensing and auctioning regards to x-ray results and hereditary diseases. Since Navicular disease today most commonly are diagnosed with radiographs, the disease can potentially be detected, selected away and prevalence reduced. The task of selection is often up to the breeding associations, and their rules for breeding will influence the hereditary diseases. For OCD there are made an emphasis on in most breeding associations already, and is cotiniously improving their selection to reduce its incidence. For navicular disease most breeding associations does not have a specific program to reduce the incident, but a general radiograph approval. The Warmblood horses have proven to have higher prevalence of navicular disease, and the four breeding associations ranked highest on the FEI world ranking list of dressage are included below.

5.1 Dutch Hanoverian (KWPN)

22 radiographs including the navicular bone front leg, coffin and fetlock on all legs, hock and stifle are required for Dutch auction and stallion licensing. Additional radiographs may be necessary upon request.

There are four classes of grading the radiographs:

- Class 0: clean/almost clean
- Class 1: good
- Class 2: sufficient
- Class 3: weak
- Class 4: bad.

OCD has its own classification (A-E) where in the fetlock no OCD is accepted, while in the stifle and hock A and B is accepted, and C under certain conditions. For the navicular bone only clean, good and sufficient x-rays are accepted by the breeding association.

In principle, the KWPN does not permit OCD in its breeding stallions. However, exceptions have been made for proven stallions, and those exceptions are now extended to younger stallions. Any exceptions are made under very strict and well-considered conditions. Screening tests for Osteochondrosis was started in 2009 to be able to approve stallions that do have an OCD but are not likely to have offspring with it when it is in some cases mostly environmental reasons. For taking part in the screening the stallion should have 20 radiographed offspring. For navicular disease they have no such program.

German Hanoverian Warmblood (HANN)

There are taken 12 pictures, not including the stifle for stallion licensing and auctions of German Hanoverian Warmblood. They classify the radiographs into four classes:

- **Class I:** Without specific abnormal radiological findings and findings categorised as anatomical variations. (Ideal condition)
- **Class II:** Findings mildly deviating from ideal condition; appearance of clinical issues estimated less than 3% in an indefinite time. (Normal condition)
- **Class III:** Findings deviating from norm condition; appearance of clinical issues estimated in 5 – 20% in an indefinite time. (Accepted condition)
- **Class IV:** Findings severely deviating from norm condition; appearance of clinical issues likely (more than 50%) (Risk condition)

To license a stallion serious x-ray findings are not allowed and symptoms indicating a hereditary susceptibility to diseases are not allowed. There are no specific comments concerning navicular disease.

Westphalian Warmblood (WESTF)

The Westphalian is a German warmblood and classifies the radiographs as the Hanoverian Breeding Association since they try to have the same standard for all German Warmblood. The question of OCD and Navicular disease are still in question by the breeding association. OCD is said to have a stable heritability in the breed, and further breeding selection are

discussed. For navicular disease they have detected a decreasing tendency. A common selection for sports horse health and not only one trait (e.g. navicular health) is their aim in breeding.

Danish Warmblood (DNW)

Stallions shall have x-rays of LM of coffin and fetlock, navicular bone front legs, hocks LM, oblique 45º, 115º and stifle LM. If changes are detected by the veterinarian in the navicular bone skyline pictures without shoes should be taken.

The radiographs are then divided into four groups;

- **Group one**: are without or with changes of no significance. Said to be normal.
- **Group two**: small changes where the clinical picture is unknown. Most likely it will not affect the presentation.
- **Group three**: significant changes with unknown effect on the presentation of the horse.
- **Group four**: Changes that most likely will have a negative affect on the horse, and are not accepted by the veterinarian.

Group three and four cannot be sold at auction, but may theoretically get licensed if it is not hereditary. As for hereditary diseases only horses with extraordinary good qualities will be accepted. The decision is done by the breeding associations' leaders.

6. DISCUSSION

Navicular disease is a common orthopedic disease among horses, and can result in premature culling or reduce in the use of the horse for sports. There are difficulties both in diagnosing and treating this disease which makes it an even bigger problem for the horses acquiring damage to the podotrochlear apparatus. The MRI is a very sensitive method to detect changes in the podotrochlear apparatus, and may detect changes that are not relevant for the horse, and that possible never cause any clinical signs in the horse. You may also get a horse with clinical signs without radiographic changes. To determine the relevance of your findings either it is x-ray, MRI, CT or any other diagnostic tool it is often evaluated both clinical signs and diagnostics findings. There are different grades of changes where the mild changes often can be detected in clinical sound horses. If we are going to reduce the prevalence of the disease a breeding selection are very useful, but if clinical signs and radiographic changes are not always following each other what should then be the criteria for breeding selection? The question whether only radiographic changes is enough to be called navicular disease is something that possibly can be solved with further knowledge of the genetic background. Most breeding associations today are focusing on healthy horses, and compulsory radiographs are used before auctions and licensing.

Screening for diseases that are not clearly understood is difficult, especially when there are several factors are involved. DNA analysis are limited when there are so many genes involved and not all are located, but some selection in breeding is still possible to do. Today the yearly auction x-rays are used for OCD screening, and the selection for OCD is already done in the breeding. OCD is a polygenetic disease that has similarities to navicular disease in point of being a prevalent disease in sports horses and both genetic and environmental influences of getting the disease. In most breeding associations radiographs are needed for licensing, and some will even refuse a stallion for breeding if it has an OCD. In Denmark and Germany the warmblood can be approved if it has especially good breeding qualities. The genetic background for OCD is widely accepted, and even radiographs will not be enough in the future, but an index value is being developed in The Netherlands. Since the genes may be carried without expression of the gene they want to reduce the breeding combinations that can result in OCD. The problem with navicular disease compared to OCD is not only the

detection that is often difficult, but the age of onset is late in life. This could possible be a solution for navicular disease as well, being a mix of environmental and genetics similar to OCD.

As it is said in the breeding association for Danish and Dutch warmblood one are in some cases allowed to breed horses with OCD if the stallion is exceptionally good. This is a problem that also makes it more difficult to get rid of genetic diseases not only OCD and navicular disease but all hereditary diseases. If a stallion carrying genes of a hereditary disease, but are a very good stallion, breeders would like to use this stallion anyway. And if it is a popular or carries very good quality performance and conformation traits the hereditary disease carriage is often overlooked.

The common aim for all studies done on navicular disease is to understand the disease and then find a way for decreasing the prevalence of its occurrence among horse population. Studies done indicate the familiar background, and with that one should be able to reduce the incidences with selective breeding. The research into which genes are involved and being able to test for it will possible be a big help. As mentioned several genes are discovered to be involved in the disease, which will make it more difficult to discover. The study into candidate genes will help understanding the pathogenesis of the disease which today is not completely understood. By understanding the way of progression and development one might be able to come up with preventative measures, or treatment that can reduce clinical signs or even stop the development before the clinical signs develop. The similarity of some of the genes found to presumably be responsible for the disease resembles the genes responsible for OA in humans. The human OA candidate genes are discovered, and they might assist in the further investigation into navicular disease.

SUMMARY

The aim of the study was to do a review of the studies regarding the genetic background of navicular disease in sports horses. Navicular disease is a common locomotor disorder in horses with a polygenetic background and when found in a horse is often causing a retirement of the horses sport carrier.

Navicular disease is a disease including several structures of the podotrochlear apparatus. The navicular bone, navicular bursa, deep digital flexor tendon, collateral sesamoid ligaments, and the distal sesamoid ligament impar are said to be involved in the disease. It is often seen in sports horses from 7-9 years of age when different degree of forelimb lameness is detected. The lameness and diagnostic nerve blocking is not the same from case to case so diagnostic imaging is the only method to prove the diagnosis. Out of these x-ray is the most common used, while MRI and CT is the most sensitive. The pathogenesis of the disease is still not completely understood but there are three theories accepted; a vascular theory, pressure from the DDFT and a process similar to Osteoarthritis. The result is the same for either theory; you have a lame horse with limited treatment procedures which mostly consists of corrective ferriary work and shoeing.

The hereditary background of the disease have been presumed for a long time by several Veterinarians and breeders, but it was first when the whole genome sequence of a horse was published in 2009 that the researches started to look for the genes involved in the disease. The heritability estimate for different degrees of radiographic changes in the navicular bone has been found to be between $h^2=0,10$ and $h^2=0,34$ by several published papers, but the heritability has been detected to be in the higher range in more severe damaged navicular bones while in mild changes the heritability is low. At Hannover University a part of the Horse Genome Project are today researching the genetics of navicular disease as a polygenetic disease.

QTLs involved in navicular disease have been found with chromosome wide significance on ECA 2, 3, 4, 10 and 26 and of them a genome wide significance on ECA 2 and ECA 10. Fine mapping of the QTL has been done on these two chromosomes with some potentially

candidate genes discovered. The candidate genes detected are similar to those responsible for human osteoarthritis.

Today there are no DNA tests or other genetic test available. In practice today there are radiographic standards horses has to pass to come into breeding, and x-rays of the navicular bone is included in these standards. Further research into the pathogenesis and which genes are involved are needed to get a better understanding of the disease for then to minimize the incidence in the horse population.

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Images:

Provided by Ansager Dyrehospital, taken by Jon Vedding Nielsen